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TECHNICAL MANUSCRIPT 287

A SERUM-FREE AUTOCLAVED MEDIUM FOR GROWTH OF ANIMAL CELLS IN SUSPENSION

Stanley C. Nagle, Jr.

MARCH 1966

UNITED STATES ARMY
BIOLOGICAL CENTER
FORT DETRICK

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Stanley C. Nagle, Jr.

Medical Bacteriology Department
BIOLOGICAL SCIENCES LABORATORY

Project 1C522301A082

March 1966

ABSTRACT

A medium, sterilizable by autoclaving, was formulated for growth of animal cells in suspension culture. The medium contains peptone, proteose peptone, glutamine, and cysteine plus the salts and glucose described by Nagle for defined media. Methylcellulose is not required. Populations of HeLa, cat kidney, and L cells of 41 x 10^5, 20 x 10^5, and 22 x 10^5 per ml were obtained after 22, 12, and 14 days of incubation, respectively. The medium provides a basis for studies leading to a heat-stable chemically defined medium. In its present form it is an easily prepared bacteriological-type medium with many possible applications in monolayer and suspended cell culture.

A SERUM-FREE AUTOCLAVED MEDIUM FOR GROWTH OF ANIMAL CELLS IN SUSPENSION

The advantages of an easily prepared, autoclavable medium for the cultivation of animal cells are obvious; however, few heat-stable media are available for cell culture. A lactalbumin hydrolyzate-yeast medium described in Merchant's Handbook of Cell and Organ Culture was unsatisfactory for our purpose. The defined medium of Nagle[1] and lactalbumin hydrolyzate suspension medium of Tribble and Higuchi[2] were not sufficiently heat-stable to permit cell growth. Recently Pumper et al.[3] reported the formulation of a partially heat-stable medium (10 lb for 10 min) consisting of a peptone dialysate and medium 199 that was used for monolayer growth of rabbit heart cells. However, the peptone dialysate must be prepared and medium 199 is a chemically defined medium containing over 60 components; this makes the preparation of an inexpensive and easily prepared medium impossible.

To prepare the medium described in this report all ingredients were dissolved together in distilled water and the solution was placed in 8- or 16-oz prescription bottles, autoclaved at 121 C for 15 min, cooled, and stored at 5 C. Approximately 1 ml of sterile 5% sodium bicarbonate per 25 ml of medium was added. More was required for high cell populations (2×10^6 to 4×10^6 per ml). Bicarbonate had been sterilized by autoclaving at 121 C for 15 min in sealed 100-ml serum bottles to minimize loss of CO_2. One hundred µg of streptomycin and 100 units of penicillin per ml were added to some experimental media.

The HeLa, cat kidney (CK), and mouse fibroblast (L) cell lines used in this laboratory have been described previously.[1] HeLa cells were used in medium-development studies.

Growth of suspension cultures in the autoclaved medium was initiated from cultures that had been growing in the defined medium of Nagle et al. (without insulin) for more than 3 years. Cultures were incubated at 35 C in rubber-stoppered 100-ml serum bottles containing 25 ml of medium in a New Brunswick Gyrotory shaker operating at 122 to 126 rpm. Numbers of viable cells were determined in the hemocytometer by the trypan blue procedure of McLimans et al.[4] Media were changed, usually on alternate days, by centrifuging the serum bottle cultures at 1000 rpm for 5 min, decanting the supernatant, and replacing it with fresh medium.

The final medium developed from these studies is shown in Table 1. Several experiments were performed to determine the necessity for each ingredient and their approximate optimum concentration. Table 2 shows the results of an experiment designed to test the possibility of simplifying the medium by omitting one of the two peptone components. Viability figures obtained after 8 days of incubation showed that it was not feasible to omit either of the peptones even though the remaining peptone

concentration was doubled. The results of a growth experiment in which the other medium components were omitted singly are shown in Table 3. The reduction or absence of growth is readily apparent in most cases. Further experiments have failed to confirm the indication given in this table that glutamine could be omitted. Table 4 shows the results of an experiment in which the concentrations of the medium components were tested at ½ and 1½ times the concentration shown in Table 1. It can be seen that the concentrations can be varied widely in some cases, though not in others; in general, the concentrations given in Table 1 (control) are quite satisfactory.

TABLE 1. AUTOCLAVABLE MEDIUM FOR GROWTH OF ANIMAL CELLS IN SUSPENSION

Component	Concentration, mg/liter
Bacto Peptone (Difco)	1000
Proteose Peptone (Difco)	1000
NaCl	7400
KCl	400
$CaCl_2 \cdot 2H_2O$	265
$MgCl_2 \cdot 6H_2O$	275
$NaH_2PO_4 \cdot H_2O$	100
Glucose	1000
Na Pyruvate	110
L-Cysteine·HCl	75
L-Glutamine	200
Phenol Red	15

TABLE 2. EFFECTS OF PEPTONE AND PROTEOSE PEPTONE ON GROWTH OF HELA CELLS IN AUTOCLAVABLE MEDIUM

Medium Variation	Viable Cells, 10^5 per ml[a/]
Peptone + Proteose Peptone (Control Medium)[b/]	8.8
Peptone	1.7
Peptone (2X concentration)	<1
Proteose Peptone	4.0
Proteose Peptone (2X concentration)	2.7

a. After 8 days of incubation. 0-hr population was 2.2×10^5 per ml.
b. Peptones used at concentrations given in Table 1.

TABLE 3. EFFECTS OF OMISSION OF SINGLE MEDIUM COMPONENTS OF AUTOCLAVABLE MEDIUM ON GROWTH OF HELA CELLS

Medium Component Variation	Viable Cells, 10^5 per ml[a/]
Control Medium (As Table 1)	13.7
Control Medium minus	
NaCl	<1
KCl	9.8
$CaCl_2 \cdot 2H_2O$	5.4
$MgCl_2 \cdot 6H_2O$	8.6
$NaH_2PO_4 \cdot H_2O$	7.4
Glucose	<1
Na Pyruvate	8.6
L-Cysteine·HCl	<1
L-Glutamine	13.1

a. After 8 days of incubation. 0-hr population was 3.7×10^5 per ml.

TABLE 4. EFFECTS OF VARIATION IN CONCENTRATION OF SINGLE MEDIUM COMPONENTS OF AUTOCLAVABLE MEDIUM ON GROWTH OF HELA CELLS

Medium Component Variation	Concentration, mg/liter	Viable Cells, 10^5 per ml[a]
Control Medium (As Table 1)		7.4
Bacto Peptone	500	6.4
	1,500	6.7
Proteose Peptone	500	4.7
	1,500	4.7
NaCl	3,700	<1
	11,100	<1
KCl	200	4.3
	600	6.8
$CaCl_2 \cdot 2H_2O$	132	3.8
	397	7.3
$MgCl_2 \cdot 6H_2O$	137	8.4
	412	6.7
$NaH_2PO_4 \cdot H_2O$	50	6.3
	150	5.7
Glucose	500	4.0
	1,500	6.5
Na Pyruvate	55	5.0
	165	7.1
L-Cysteine·HCl	37	4.3
	112	3.2
L-Glutamine	100	5.7
	300	3.5

a. After 8 days of incubation. 0-hr population was 2.4×10^5 per ml.

Growth curves of HeLa, L, and CK cells in the autoclaved medium are shown in Figure 1. Media were changed daily for these tests. Maximum yields were obtained after 22, 14, and 12 days of incubation for HeLa, L, and CK cells, respectively. Growth of these cells in the autoclaved medium is generally comparable to that obtained in the chemically defined medium. However, CK and L cells usually give higher maximum cell counts and HeLa cells give lower maximum cell counts in the defined medium. This growth phenomenon is reversed in the autoclaved medium probably because HeLa cells were used in the development of the autoclaved medium, whereas CK cells were used in the defined medium studies.

The autoclaved medium described in this report is an easily prepared bacteriological-type medium and should have many applications in monolayer and suspended cell culture. Preliminary studies have indicated that, in addition to its use in suspension cell culture, the medium when supplemented with 5% fetal bovine serum allows the growth of epithelioid and fibroblastic cells as well as diploid cell strains in monolayer culture. In addition, the medium should provide a basis for studies leading to a heat-stable chemically defined medium.

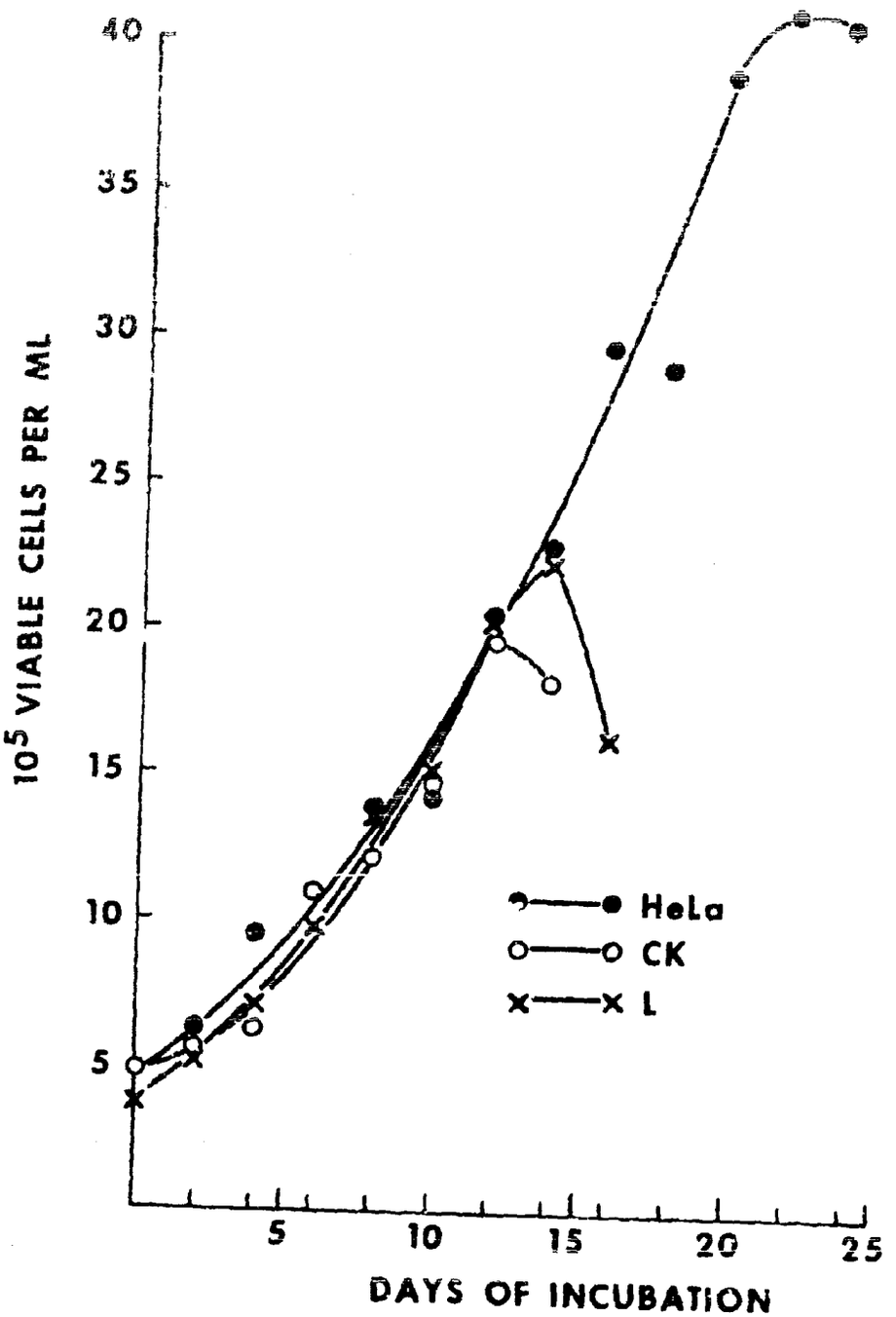

Figure 1. Growth of HeLa, CK, and L Cells in Autoclavable Medium.

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